UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

001761

4/18/1982

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE:

SUBJECT: Review of IBT Replacement Data on Sencor® EPA. No. 3125-270. Acc. No. 246397. Caswell No. 33 D.

FROM: Roger Gardner, Toxicologist
Review Section IV
Toxicology Branch, HED (TS-769)

Roger Gardner C.F. Chasson 4/18/82

TO: Robert J. Taylor
Product Manager (25)
Registration Division (TS-767)

Action Requested

Review of a two-year mouse feeding study and a teratogenicity study in rabbits.

Background

The two above mentioned studies have been submitted by Mobay Chemical Corporation on November 18, 1981 to replace similar studies conducted by Industrial Bio Test. In a memorandum dated December 16, 1980 to Jan Auerbach of the Lab Audits and Regulatory Analysis Branch (SPRD), Gary Burin of the Toxicology Branch stated that those two IBT studies were classified by the Canadian Government as invalid.

Recommendation

Both studies are acceptable as replacements for the IBT studies.

Citation: Hayes, R. H. 1981. Metribuzin (Sencor®) oncogenicity study in mice. Study No. 78 CCM01. Mobay Chemical Corporation. Stilwell, Kansas. EPA Acc. No. 246397.

[redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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[redacted] INGREDIENT INFORMATION IS NOT INCLUDED

Materials and Methods: Test substance: The test substance is identified as the technical grade (92.9% active ingredient) of metribuzin (Sencor®). The chemical name is 4-amino-6-(1, 1dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one. Contaminants include the following:

[redacted]

The remaining constituents included [redacted] The test substance was a white powder (Batch No. [redacted]-297-50).

Test Species: Outbred CD 1, male and female mice from Charles River Laboratories were used. These animals were 28 days old when received and 46 days old at the beginning of the experiment. Food and water were provided ad libitum.

Procedure: Groups of 50 male and 50 female mice were given diets containing 0, 200, 800, or 3200 ppm metribuzin. Based on food consumption and analytical chemistry results the authors stated that these concentrations corresponded to daily doses of 0, 28, 111, or 435 mg/kg body weight for males and 0, 35, 139, or 567 mg/kg/day in females. Test diets were provided for 104 weeks. The test chemical was dissolved in corn oil, and the solution was added to Purina Rodent Chow No. 5002-4 (minus 1% fat) so that the total fat content was 1%.

Animals were observed for signs of toxicity or mortality twice each weekday and once a day during weekends and on holidays. Each animal was palpated for masses once each week, and body weights and feed consumption were also determined weekly. Blood was drawn from the orbital venus sinus of 10 mice of each sex which were randomly selected from each group. The blood was used to determine hematocrit, hemoglobin, erythrocyte counts as well as total and differential white cell counts at 6, 12, 18 and 24 months. At 24 months the animals were sacrificed and necropsied. At necropsy the liver, kidneys, lungs, gonads, spleen, brain and adrenals were weighed.

[redacted] INFORMATION IS NOT INCLUDED

Histological examinations were done on the following tissues:

brain
eyes
harderian gland
pituitary
salivary gland
heart
thymus
thyroid with paura-thyroid
trachea
vertabrae
spinal cord
nasal cavity
esophagus
small intestine
large intestine
adrenals
pancreas
liver
gallbladder
kidneys
urinary bladder
spleen
paranasal
tongue
oral cavity
lymph nodes
testes
prostate
ovaries
uterus
mammary glands
Sciatic nerve and skeletal-muscle
Sternum and bone marrow
nasopharynx
middle ear
all gross lessions

Microscopic examinations of grossly enlarged nodes and nodes draining known or suspected tumor sites were also done.

Statistical analyses of body and organ weight data as well as feed consumption included analysis of variance, test of Least Significant Difference, and Duncan's New Multiple Range test.

Reported Results;

Mortality was 58% in males and females of the control and high dose groups and in the low dose males by the end of the experiment. The low and mid-dose females had respective mortalities of 48 and 56%, while the males of the mid-dose group were reported to have 36% mortality by the end of the study. Mean survival times were 631, 642, 657, and 662 days for males in the control, low, mid, and high dose groups, respectively. The respective values for females in those groups were reported as 601, 661, 616, and 609 days.

Body weights were found to be comparable with controls for males in treated groups. Female mice receiving the 800 ppm diet showed statistically significant increases of 6 to 7% in mean body weight at various times during the first 6 months, while all other reported weights were comparable to those of untreated female mice.

Hematology results showed statistically significant differences that were sporadic according to the author. Hematocrit and hemoglobin results in females of the high dose group were 13% lower than those determinations in untreated female mice at 24 months. The hematocrit for females in the mid-dose group was also 13 % less than the control value at the end of the study.

Although no historical or referenced data were provided, the authors stated that hemoglobin and hematocrit values (11.3 g/dl and 33.9%, respectively) were below the expected range for female mice. Decreased erythrocyte count and hematocrit were also noted at 18 months in the 3200 ppm group of female mice. The respective decreases were 13% and 10% from control values. The author concluded that these results were dose-related. No other hematological parameters were considered to be affected by treatment.

The mean absolute liver weight in females of the high dose group was 3.131 g which was found by the investigator to be statistically significantly greater than that of control females (2.132 g). Kidney weights for these mice were also significantly increased (0.711g in treated mice and 0.628 g in controls). The only other statistically significant difference between control and treated groups with respect to mean absolute organ weights was an increased liver weight in the males receiving the 800 ppm diet (2.978 g in the treated males and 2.505 g for control males).

Relative organ weights (expressed as percent of body weight) for the liver in all treated groups of female mice were statistically significantly increased from that for control females. The results for the control, low, mid, and high dose groups were reported as 6.281, 7.496, 7.519, and 9.415%, respectively. These values for the spleen and kidneys in high dose females were also increased (1.395 compared with 0.901 for the spleen of treated and control females, respectively; and 2.149 compared with 1.922% for kidneys in treated and control animals respectively). The only other relative organ weight reported as significantly different from control values was in the male mice given the mid-dose level. Treated males had a relative liver weight of 7.850% compared with 6.738% in control males.

In animals that died during the first 18 months the author noted that tissue masses resulted from lymphoreticular proliferation in the spleen, lymph nodes, and thymus. Female mice were found to have cysts in the ovaries and uterine endometrium. Amyloidosis was also noted as a common lesion, and the author stated that this lesion was manifested by tan kidneys, fluid filled intestines and edema. All of these lesions were seen in all groups at comparable incidences. They were not attributed by the investigator to treatment of mice with the test substance.

Mice sacrificed during the remainder or at termination of the study were reported to have gross lesions most frequently in the liver and lung. The author characterized these lesions as the type which are expected after 20 months of age in the strain of mice used, but no reference data were provided in support of that conclusion.

Common histopathological non-neoplastic lesions which were reported included glandular hyperplasia and chronic inflammation of the gastric mucosa and amyloidosis. The latter lesion was slightly increased in males receiving the 3200 ppm diet according to the authors, but the incidence was considered to be within expected range. The authors did not provide data to support that conclusion. All groups had comparable incidences of gastric mucosal inflammation. These observations were not considered to be compound related.

Malignant lymphoma, hepatocellular neoplasms, and alveolar-bronchial carcinomas were the most frequently observed tumor types. The lymphomas were described by the author as multicentric lymphosarcomas. They were found in 20 to 26% of the female mice in each group and 6% (high dose group) to 26% (control group) in males of each group. Hepatocellular neoplasms (adenocarcinomas and adenomas) were reported to occur almost exclusively in male mice. The reported incidences for each group were from 7% (high dose group) to 25% (control group).

The incidence of pulmonary neoplasms were reported to be from 20% in control males to 6% in the high dose males, and the incidences in females were reported to range from 2% in the high dose group to 22% in the low and mid dose groups (the female control group had a 14% incidence). With respect to the lung tumors the authors stated that the incidence is within normal limits for inbred strains of mice, and that for the high dose group females is below normal ranges. However, no data to support this conclusion were provided (see discussion below).

Other tumor types were noted, and the incidences were 8% (4 of 50 animals) or less for a given tumor type in any one group. Hardarian gland adenocarcinoma, mammary gland adenocarcinoma, rhabdomyosarcoma, cholangiofibroma, hemangioma, hemangiosarcoma, islet cell adenoma in the pancreas, follicular adenoma in the thyroid gland, renal cortical adenomas and carcinomas, transitional cell carcinoma in the urinary bladder, interstitail cell adenoma in the testes, pheochromocytoma, pituitary adenoma, basal cell carcinoma in the skin, aleiomyoma and adenocarcinoma in the uterus, and leiomyosarcoma were the other tumors reported. None of these tumors were observed to occur in a dose related manner.

The author stated that the criteria for diagnosis of malignant and benign lesions proposed by Houlton (1978) were used. Benign tumors were characterized by the authors as growing by expansion within limits (cirumscribed or encapsulated) and without metastasis and rarely invading vessels and other structures. Tumors were also diagnosed as benign if their cells were differentiated, uniform in size, and rarely showed mitotic figures. In addition, the authors considered tumors benign if there was usually abundant stroma with adequate blood supply and minimal tissue destruction which, if present, is characteristic of space-occupying lesions. The criteria used to diagnose malignant tumors included unlimited growth by expansion or infiltration with frequent metastasis and invasion of vessels and other structures. The authors also considered tumors malignant if mitotic figures were frequent, and cells were pleomorphic, hyperchromatic, imperfectly differentiated, and had large nucleoli. Malignant tumors were also diagnosed when these criteria were noted along with scanty stroma and when the blood supply was outgrown by the tumor.

The results (expressed as number of malignant tumors per number of total tumors diagnosed) are summarized as follows:

Group	Males	Females
Control	85/93	56/61
Low dose	39/48	88/94
Mid dose	40/47	61/75
High dose	33/40	69/76

The reported number of tumor bearing animals for each group of 50 mice is summarized as follows:

Group	Males	Females
Control	28	19
Low dose	26	28
Mid dose	22	29
High dose	18	20

The number of animals bearing malignant and benign tumors was reported as follows:

Group	No. with malignant tumors	No. with benign tumors
Males		
Control	23	9
Low dose	20	9
Mid dose	16	7
High dose	13	6
Females		
Control	16	4
Low dose	24	6
Mid dose	21	11
High dose	15	5

(It should be noted that the two categories listed here are not mutually exclusive. An animal can have benign and malignant tumors, malignant tumors, benign tumors, or no tumors.)

Discussion:

Although the author did not report statistical analyses of tumor incidence data, an independent analysis using the Chi square test has been conducted for those treatment groups which appeared to be markedly different from the appropriate control group. Mid and high dose groups of male mice showed a decrease in the number of malignant tumor bearing animals. The decreased incidence in the high dose group was statistically significant ($p = 0.037$), while that for the mid dose group was not ($p = 0.151$). The decreased number of tumor bearing animals in the high dose group below that of control male mice was statistically significant ($p = 0.045$).

When these tests were applied to reported increased incidences of tumor in treated female mice the following results were obtained:

Group	Tumor Type	p value
Low dose	tumor bearing animals	0.071
Mid dose	malignant tumors	0.045
Mid dose	benign tumors	0.0499

Comparisons of the incidence of lymphomas, hepatocellular adenocarcinomas or adenomas, and pulmonary tumors showed that observed decreases were statistically significant. These findings are summarized as follows:

Group	Tumor Types	p value
Low dose (males)	malignant lymphoma	0.037
High does (males)	malignant lymphoma	0.006
High dose (males	hepatocellular adeno-carcinoma	0.053
Mid dose (males)	lung tumors	0.037

No statistically significant differences between control and treated groups were found for any other reported incidences.

These statistical comparisons suggest a consistent dose dependent trend for tumor incidence in male mice. The number of tumor bearing females was increased in the low and mid dose groups along with an incidence in the high dose group which was comparable to that in control female mice. No other consistent dose-related trends were found.

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Decreased tumor incidences in treated groups of males and the high dose females are not likely to result from mortality as shown by group mean survival times discussed above. In addition, reported cumulative mortality data show that only at 18 months is there increased mortality in treated groups above that in controls. These increases were noted only in the high dose groups. These data are summarized as follows:

Group	Males	Females
Control	13/50	13/50
High dose	15/50	17/50

Independent statistical analysis of these results (Chi square test) showed that the high dose groups were not significantly different from their control groups. Reported data on the incidences of the most frequently observed tumors (lymphomas, liver cell tumors, and lung tumors) were examined with respect to mortality in order to determine how early mortality affected those results. The only groups in which animals were reported dead before the first liver tumors were diagnosed were those of the mid and high dose groups of males. These early deaths had no effect on the incidence data reported for lymphomas or lung tumors as well as the number of tumor bearing animals for those groups. These considerations show that decreases in tumor incidence reported for treated groups of male mice and the high dose group of female mice are not the result of mortality.

In those groups reported to have increased tumor incidences, the author stated that these results were within normal ranges for aging mice. As mentioned in the previous section no data were provided in support of this statement. A compilation of data on the spontaneous incidence of tumors presented by Mitruka et al (1976) shows that liver tumors occur at rates from 0 to 57% for 17 inbred strains of mice. The range reported by Mitruka for lung tumors is from 2 to 65%. When incidences are combined for all 17 strains the ranges are 4 to 8 and 5 to 9% for liver and lung tumors, respectively. Since the strain used in the study reviewed here was described as an outbred strain, the combined data are also appropriate for comparisons. In addition, Bickerton (1973) reported that the incidences of lung tumors, hepatomas, and reticulum cell sarcomas in wild female mice are 12, 2, and 3%, respectively, while those respective values for wild male mice are 5, 9, and 16%. These data support the author's conclusion that observed increases in tumor incidence are within ranges noted for other strains of mice.

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The lack of a consistent dose related increase in tumor incidence, the similar survival rates in treated and control groups considered with decreases in some tumor incidences, and the similarities between observed tumor incidence in treated mice and ranges of incidence in other strains diminished the toxicological significance of increases noted in numbers of tumor bearing animals, malignant tumors and benign tumors.

Although mortality and body weight data do not indicate that the highest dose had toxic effects, hematocrit, hemoglobin, and liver weight data suggested that the 3200 ppm diet (435 mg metribuzin per kg body weight in male mice and 567 mg/kg for female mice) caused minimally toxic effects.

In view of these considerations the study demonstrates that under the test conditions metribuzin does not increase the incidence of tumors in mice. However, the significance of the apparent increases in tumor incidences noted above must be evaluated in the context of results from a long-term feeding study in a second species before they can be finally dismissed as coincidental findings.

References:

Bickerton, R.K. 1973. Spontaneous tumors and related pathologic changes. Section 11. In. Goldberg, L. ed. Carcinogenesis Testing of Chemicals. CRC Press, Inc., Cleveland, Ohio.

Houlton, J.E. 1978. Tumors in Domestic Animals. Second Ed. University of California Press. Berkeley, California.

Mitruka, B.M., H.M. Rawnsley, and D.V. Vadehr. 1976. Animal models in Gerontology. Ch 12. In Animals for Medical Research: Models for the study of human diseases. John Wiley Sons. New York.

Core Classification: Guideline

Citation: Unger, T.M., and T.E. Shellenberger. 1981. A teratological evaluation of Sencor® in mated female rabbits. (Unpublished report prepared by Midwest Research Institute). Submitted to the EPA by Mobay Chemical Corporation, Environmental Health Center. Stilwell, Kansas. EPA Acc. No. 246397.

Materials and Methods:

Test Substance: A sample of technical grade metribuzin containing 93% active ingredient was used. The test material was a composite of 5 batches.

Test species: Five-month old virgin female New Zealand White rabbits were used. They were housed individually with a male rabbit and observed for mating behavior prior to the start of the test. If the first mating was successful (mounting by the male was the indicator), the female was placed with a second male. Those females which were successfully mounted twice were considered mated. The authors noted that the second pairings for some of the rabbits failed, but those rabbits were included in the test. This procedure was conducted with enough rabbits to assure 19 to 20 mated females with anticipated pregnancies in 13 to 15 animals for each control and treated group of rabbits. The day of mating was considered day 0 of gestation.

Experimental procedure: A solution of 0.2% K-4M Methocel and 0.4% Tween 80 was used as the vehicle for administration of the test subtance to three groups (see the previous paragraph for description of group sizes), and a fourth group was included as the vehicle control. Doses were administered by gavage on days 6 through 18 of gestation and were 0, 15, 45, or 135 mg/kg/day.

The test animals were observed daily for signs of toxicity. Maternal body weights were determined on day 0, 6, 13, 18, and 20 as well as the day of terminal sacrifice. On day 30 of gestation the does were sacrificed and examined grossly for external and internal lesions. The uterus was removed from each doe and examined to determine the numbr and position of fetuses. Corpora lutea, resorption sites (early and late) and implantation sites were counted.

Fetuses were checked for viability and weighed. Half of the fetuses of each litter were examined by the freehand technique of Wilson. The remaining fetuses were fixed and stained for skeletal examination.

Data were first evaluated by Bartlett's test for homogeneity. Homegeneous data were analyzed by Dunnett's or Tukey's Omega procedures, while heterogeneous data were evaluated by a nonparametric rank test. Differences were considered statistically significant when p values were less than 0.05.

Reported results: The authors reported an overall pregnancy rate of 84.8% with one doe in the control group and 2 in the high dose group whose litters were completely resorbed. One doe each was lost from the low and mid dose groups because of early delivery which was the result of miscalculation of mating. Dosing errors were reported to cause the deaths of one doe from each of the control, low and high dose groups, while pneumonitis was cited as the cause of death in two control, one low dose and one high dose rabbits. One doe from the control group and one in the low dose group died of unknown causes.

Abortions were reported for one of 17 does in the control and one of 17 in the mid dose group, while the high dose group had 4 aborted from 16 pregnancies. A decrease in feed and water consumption as well as decreases in fecal and urinary output were noted but quantitative results were not presented. The authors stated that these decreases were dose related.

Maternal body weights for the control, low and mid dose groups were not statistically significantly different. Body weights for the high dose group were decreased by 9, 13, 12, and 5% from control values on days 13, 18, 20, and 30, respectively. No differences were noted for these two groups before treatment began.

No significant differences between control and treated groups were reported for the mean numbers of corpora lutea, implantation sites, early or late resorptions, live fetus or dead fetuses in each doe.

Respective mean fetal weights for the mid and high dose groups were 9 and 14% less than that of the control group.

The authors stated that fetuses having a weight of 30g or less in litters where few of the other fetuses had a similar weight were judged to be undersized. By this criterion 9 of 15 litters in the mid dose group contained undersized fetuses. There were 24 undersized fetuses among the 136 in that group. The remaining treatment groups were comparable to controls with respect to incidence of undersized fetuses.

Gross anomalies were noted to occur in one to two pups of one or two litters in each group. These anomalies were reported to be scattered among the test groups with no relationship to treatment. One pup of one litter in the high dose group had a protruding tongue. Another pup from a litter in the mid dose group had a cleft palate and a missing limb. Other gross anomalies were reported, but they occurred with a similar incidence and will not be discussed here.

The most frequently observed soft tissue anomaly was abdominal hemorrhage which was noted to have the same incidence in all groups. Other anomalies occurred in one or two pups from one or two litters and were not attributed by the authors to the test substance.

Most of the skeletal lesions were described as incomplete ossification, fused, improperly aligned, or split bones principally involving the sternebrae. The incidence of these and other skeletal anomalies was reported by the authors to occur in a pattern similar to those of gross and soft tissue anomalies.

Discussion: The investigators concluded that under the conditions of their study Sencor® caused maternal toxicity without significant fetal toxicity at the high dose (135 mg/kg/day). They further stated that no maternal or fetal effects were found at the low dose (15 mg/kg/day). The investigators also concluded that Sencor® is not fetotoxic or teratogenic in rabbtis.

The authors' conclusions are generally supported by the results of the experiment. The study supports a no-effect level for maternal toxicity and fetal effects of 15 mg/kg and a lowest effect level for maternal toxicity of 45 mg/kg/day.

Core Classification: Guideline